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EXPERIMENTAL DIFFICULTIES WITH THE MEMORY CODE

There has been a tendency recently to consider the invertebrates[1-9] and even bacteria[10] as possible ideal systems for lnding the nature of the experiential memory code. Which invertebrate is ideal depends, of course, on what chemical and physical experimental techniques are used. Some organisms are too small for electrical recording from neurons or for chemical analysis of one organism at a time. Others are too large to survey the entire nervous system in looking for morphological changes accompanying memory or in recording from a significant number of the total number of neurons that may be active in the learning process. Since there is probably no organism perfectly suited to all of the techniques available, the kind of organism studied should be optimally compatible with the particular technique that is to be used. The absolutely simplest organism may not be at all simple or practical to use with a given technique.

There have been reviews of the literature on the biological nature of learning and memory[11-15] but little analysis of the different ways in which the memory code may be detected. The code must be chemical and/or physical, of course, and this covers all possibilities. But for convenience, memory will be considered as being either chemical (involving composition changes in molecules), structural (conformational changes of molecules), or electrical (changes in flow of charged particles), or some combination of these. Within this framework an evaluation will be made of the experimental approaches most relevant to finding a memory code.

AN ELECTRICAL CODE

If incoming information from the environment could develop electrical reverberating circuits in the nervous system[16,17] that lasted for periods of time consistent with what psychologists know about memory spans, different neural circuits could contain different memory traces. There is no evidence that a repeating pattern of electrical activity directly represents a memory trace, but there is some evidence that reverberatory circuits can at least exist. Cortical slabs isolated from the brain of a cat showed, when stimulated briefly, recurrent bursts of electrical activity lasting about thirty minutes.[18] Upon sensory stimulation of the thalamus of a cat, repeating pat-

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terns of unit discharge were detected by microelectrodes and this pattern changed when the stimulus did.[19] These recurrent patterns last a relatively short time; therefore, such a phenomenon, if it exists for memory, must be restricted to short-term memory,[20,21] defined as a period of time during which memory is very labile and more subject to disruption by external agents (such as drugs) than at a later time. When memory becomes more resistant, it is considered consolidated and, hence, long-term. A well-known external agent that affects short-term memory is electroconvulsive shock (ECS). When given to animals soon after a learning experience, the animals forget the task, but if it is given much later, their memories are not affected nearly as much, if at all.[22] The interference with the consolidation process is thought to be permanent. One group of researchers[23] found that when rats which had been given ECS immediately after learning and had exhibited memory loss were retested 48-72 hours later, they showed memory recovery. However, a replication of this experiment did find memory loss to be permanent following ECS[24] and another experiment demonstrated that the phenomenon of memory loss can be a function of the method of testing.[25]

The relevance of ECS to a theory of reverberatory circuits is that it is considered to destroy or interfere directly with any existing electrical memory circuitry.[12,20,26] However, ECS decreases RNA synthesis[27,28] and increases norepinephrine synthesis.[29] Electrical stimulation of rat brain slices *in vitro* also decreases RNA plus protein synthesis.[30,31] If hamsters are kept in the cold after learning and are given ECS, there is more interference with memory than when the animals are kept at room temperature before ECS.[32] Memory in mice given strychnine after learning is unaffected by ECS 10 minutes later, while control groups lose the memory after ECS.[33] All these results indicate that ECS disrupts memory not only by adding electrical noise to any reverberatory circuits that may exist but also by causing biochemical changes. These chemical effects have not been shown to act directly on those chemical events that presumably generate the electrical reverberating circuits and it is therefore not possible to say that ECS has anything to do with reverberatory circuits. Furthermore, these circuits have not been shown to exist for memory and so one cannot claim that ECS eliminates them.

Experiments have been performed where animals that are cooled after learning (to temperatures as low as 0°C. for rats) show no major memory loss when returned to room temperature.[34-36] These experiments have been taken to indicate that since there is "gross electrical silence" of the brain at these low temperatures, neuronal reverberatory circuits cannot exist.[37] This conclusion would not necessarily apply to short-term memory since

it takes about one hour to reach these low temperatures and reverberatory circuits may be present for only a short time.

The direct way to look for such memory circuits is to use electrical recording techniques. Recording microelectrodes can be placed in various parts of an animal's nervous system to determine what neuronal pathways are active during learning and memory, and then an analysis of the recordings can be made to determine if some code is present. This search for coding would involve a detailed analysis of the action potentials with regard to waveform, amplitude, and frequency. Methods exist for waveform analysis,[38] but present thinking assumes that, for the action potential, "no information content in the mathematical sense appears to be carried by the form of the spike discharge itself."[39] The all-or-none feature of action potentials in axons implies no amplitude differences, but there are exceptions to this.[40] There can be time differences, of course, between each spike and hence frequency changes along an axon. For graded potentials, there can also be waveform and amplitude differences as well as frequency ones. Techniques are available to permit analysis of these parameters[41,42] but no coding of memory using these parameters has as yet been discovered. Incoming information from the environment is coded in various ways by different sensory receptors, but whether existing spontaneous electrical activity serves as a carrier to be modulated by the information[43] or whether the action or gradient potentials serve directly as the memory carrier is not known. However, the F.M. information capacity of nerve cells has been calculated.*

The experimental problem is to find an organism or an isolated neural preparation that exhibits learning and memory and that allows electrical recording from all those cells active in the learning. The best preparations in this regard come from the cockroach, the locust, and the mollusk *Aplysia*. A headless cockroach can be habituated to a repeated puff of air applied to its anal cerci so that upon repetition of the puff, the insect no longer jumps in response to it.[7] This habituation takes place in the last abdominal ganglion which connects with the cercal nerves. A repeating pattern of electrical stimulation applied directly to the cercal nerve also produces habituation. However, another stimulus of the same intensity, but different in frequency, applied to this habituated nerve elicits a response,[44] indicating the response is very specific to the stimulus used, and does not involve gross sensory adaptation to it or fatigue. *Aplysia* has been habituated to drops of water falling on its head so that it no longer withdraws its tentacles upon presenta-

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* R. B. Stein: The information capacity of nerve cells using a frequency code. *Biophys. J.*, 1967, *7*, 747.

tion of the stimulus.[2] The recordings from a giant cell in the abdominal ganglion indicated that the excitatory post synaptic potential decreased in magnitude as the animal became habituated. The fact that this giant cell is not located in the "habituation reflex arc" shows that habituation was not just a local peripheral phenomenon.

In a locust, a mesothoracic ganglion and a nerve connecting it to an adductor muscle can be isolated and kept alive in saline solution.[6] This preparation shows conditioning. Whenever the frequency of discharge, as measured by electrodes, along the nerve decreased, the muscle was touched and the frequency of discharge by the ganglion increased. Eventually, a higher frequency of impulses was maintained along the nerve without further touching. Another isolated preparation has been obtained from *Aplysia*:[8] using an abdominal ganglion, recordings were taken from one of its giant cells. When one of the ganglion's nerves was given an excitatory input of small intensity (producing only a small post-synaptic potential in the giant cell) and then was paired with a large intensity input to another nerve (producing a larger post-synaptic potential in the giant cell), it was found that the weaker stimulus given alone eventually produced a larger post-synaptic potential than before pairing. This result meets the definition of classical conditioning and is called "heterosynaptic facilitation." However, this may not be the same phenomenon as that seen when environmental stimuli produce the conditioning. While stimuli from the organism's environment reach this ganglion via electrical activity, as does the direct electrical stimulation of it, the amount and kind of information transmitted could be expected to differ because of the variations in electrical activity generated.

All these preparations involve ganglia with a countable and, perhaps, fixed number of cells. They offer, at present, the simplest systems showing some form of learning from which electrical recordings can be made inside identifiable neurons. Although organisms exist that are smaller in size than these preparations and that show learning,[1] they are too small to record from; another mollusk preparation with identifiable cells that can be recorded from has not yet been shown to exhibit learning.[45] To be looked for are electrical signals corresponding to the learning; this involves recording from those cells active during the learning process. But the *Aplysia* ganglion contains about 1,000 cells and the cockroach and locust ganglia contain a few hundred cells. It would be very difficult to record from all these cells to localize those involved in the learning, since some of them are quite small and/or are difficult to reach without disturbing other cells. Recording from only a portion of the relevant cells may miss the integrative nature of the phenomenon and hence any memory coding. The situation is much better,

of course, if only a few cells in the ganglion are involved in the learning process. Even in this simplest case, the difficulty in detecting an electrical code may lie in the nature of the action and graded potentials. If the "sodium pump" theory of nerve conduction is correct, the electrical events recorded become the result of underlying chemical changes, which may not even develop an electrical code based on action and graded potentials.

As Katz[46] has suggested, "our preoccupation with rapid electrical methods . . . has not enabled us to find a way of approach to the great problems of long-term interactions and modifications in the nervous system." These methods may be of use in short-term memory but for long-term memory, structural and chemical effects have received the most attention.

A STRUCTURAL CODE

Any structural change accompanying learning and memory would presumably be quite small, otherwise there would be interference with normal cellular metabolism. There is no compelling *a priori* reason why there should or should not be such changes in place of or in addition to chemical changes. However, structural theories concerning changes around synapses due to stimulation effects have been proposed. These have been reviewed by Eccles[47] and more recently with extensions by Aidley.[48] In the early part of this century there was a report[49] that the size and shape of Purkinje cells from a dog's cerebellum changed after the animal had been exercised in a treadmill, but this was later discounted.[50] Edstrom[51] has reviewed three very early papers that claimed an increase in neuronal volume due to an animal's increased activity and three that found no such increases. He reported from his own work an increase in soma volume of spinal cord neurons in guinea-pigs following short-term motor activity, but suggests this may be the result of increased water uptake. He did find, though, a nucleolus volume increase as a result of long-term motor activity and suggested this increase may indicate a rise in protein synthesis. A reduction in the size of synaptic vesicles in the rod and cone regions of rabbits kept in the dark for several days has been reported,[52] only to be discounted later.[53] In comparing a group of rats kept in the dark with an experimental group kept in light and given sound stimulation, it was found that polysomes increased in the experimental group as did protein synthesis.[54] It has been shown that stimulation at 100 pulses per second of a rabbit's nerve increases the number of synaptic vesicles found per unit area when compared to controls.[55] With more intense stimulation (400 pulses/sec.) the number of vesicles is reduced, compared to controls, and this is attributed to fatigue. A focal electric field applied to fibroblast and neuronal cells in culture causes a

local deformation of the membrane.[56] In the mouse cerebellum there appears to be a decrease in size and an increase in elongation of vesicles as the animal ages.[57] There is even evidence of postnatal neurogenesis of microneurons in rats.[58] This may represent premature birth of the animals before the cells are fully differentiated and formed, or may result from sensory maturation due to environmental feedback. Rats raised in an "enriched" environment (where they are subjected to a wide variety of stimuli) compared to their litter mates raised in an "impoverished" environment (subjected to minimal environmental stimuli) show an increase in the weight and thickness of their cerebral cortex,[59] an increase in glial cell counts, and possibly in the size of neurons.[60] Enriched environments also increase glial cell multiplication in the adult rat.[61] None of these papers has shown a structural change resulting from learning and memory alone, though the "enriched versus impoverished" experiments come the closest. The differences between the two groups in the stimuli received and their activity exhibited are compounded with any learning differences that may have occurred, and it is not possible to say that the anatomical changes found reflect learning differences specifically. The plasticity in the nervous system found in many studies suggests, though, that memory could have a structural basis.

Since we do not know where in the nervous system to look for structural memory changes, we must survey the entire system involved in the learning. Again, a "simple" system is needed. The cockroach, locust, and *Aplysia* preparations qualify, as do the micrometazoa. These latter freshwater animals are all less than 1 mm. in size, with a correspondingly smaller nervous system than the ganglia of the cockroach, locust, and *Aplysia*. The further advantage is that the whole animal can be trained by standard psychological learning procedures. This training of whole animals circumvents the difficulties that may arise in trying to train isolated nervous systems. It may be, however, that though the micrometazoa have smaller nervous systems, they may be more complicated ones than those of the isolated preparations, which surely perform fewer functions than the nervous system of an entire animal. The isolated ganglia mentioned contain too many cells to monitor with the electron microscope in looking for structural changes. However, if it can be shown that only a few cells are involved in the learning, these ganglia become feasible systems. Unfortunately, stimulating individual nerve cells in culture and looking for structural or chemical changes is probably irrelevant to the question of what happens during memory since the form and content of this input differs from environmental input.

What needs to be shown is that a particular nervous system, either intact or isolated and capable of learning, has the same ultrastructure from animal to animal so that a trained one can be compared to an untrained

one. If the variation is too great between members of the same species, the individual differences will make it clearly impossible to detect any ultrastructure memory effects. The protozoa, because of their relatively large behavioral repertoire,[62] should not be ruled out as organisms in which to look for such changes, although at present there is doubt about the ability to demonstrate learning in them.[9] A nervous system may not be necessary for learning and may only be an evolutionary improvement to facilitate the process in higher animals.

Given a system where it is feasible to look for morphological changes accompanying learning, can the changes be detected? The answer will come in the looking, of course. But if the changes involve the size and shape of synaptic vesicles, changes along synaptic clefts, or local deformations of neuronal membranes, etc., there will be great difficulty in detecting them. It is difficult enough to orient the specimens so that sections can be made in the same plane for both the control and experimental groups, for differences in the plane of sectioning will obscure any potential structural changes. The changes could also be below the resolving power of the electron microscope. There is, for example, no way of determining the orientation of molecules with respect to, say, a membrane following learning. If changes are detected, then what? They may merely reflect a change in the transmission characteristics of the nervous system and may reveal nothing of an underlying memory code. However, the form of the structural changes may be the code itself, or the structural changes may contain a chemical code within themselves. Which, if any, of these possibilities exists is not at all certain.

A CHEMICAL CODE

Most of the research on the memory code has centered around chemical approaches, probably because we know more cell chemistry than physics and because we know of the existence of a coding process in the nucleic acids. It is not surprising, therefore, that a large body of the work in the chemistry of learning has centered on the nucleic acids and proteins. It would be surprising if they were not involved in some way with learning and memory since they are key substances in all cells. Because protein and RNA synthesis vary with the stimulation an animal receives and with its activity,[12,63] proper controls are needed to separate these changes from any that may result from learning and memory. Control groups should do everything the experimental groups do except learn, otherwise the differences in activity and environmental stimulation received will produce protein and RNA changes over and above any that may occur during learning. This

experimental procedure can pose a contradiction, since if two sets of animals are given the same stimuli and undergo the same activity and one set learns and the other does not, there is considerable doubt that learning is really going on. However, by changing the temporal presentation of the stimuli, this problem can be mitigated for some forms of learning. In classical conditioning, for example, the controls can be given the unconditioned stimulus and conditioned stimulus in reverse order from the experimental group or they can be given them at random. One of the better controlled experiments is that by Zemp, *et al.*[64] but even here, as the authors point out, there is a locomotor activity difference between controls and experimentals that could account for the detected RNA synthesis differences between those that learn the avoidance task and those that do not. Since avoidance training has some stress associated with it, which could produce chemical changes in hormone levels, as has been detected in the monkey,[65] stress must be controlled for as well. Activity differences may have an effect on the experimental results apart from any effect on synthesis rates. For example, the drug magnesium pemoline was shown to facilitate some kinds of learning[66] when compared to controls given saline. But a closer examination indicated that this drug may have increased spontaneous activity and that the apparent learning advantage in the drugged animals could be attributed to this.[67] The experiments by Hyden[68-71] are also subject to criticism in the handling of controls in terms of sampling and activity differences. The individual cells taken for analysis from the experimental group were not (and perhaps could never be in a brain the size of a rat) the same cells in terms of their exact location in the brain as those taken from a control group,[72] so that the chemical differences reported may be spurious. There are also obvious stimulation differences between experimentals and controls[13] and it must be said that the chemical differences reported reflect a compounding of sampling, stimulation, and learning effects. An experiment with planaria[13] substantiates these conclusions. Base ratio changes in RNA were found between an experimental group that received light and shock with the head toward the cathode and two control groups, one of which was not exposed to the stimuli and the other of which was given light and shock only when its head was toward the anode. Neither of these controls learned the response. However, these same base ratio differences found in the experimentals were found in other controls that received either random light paired with random anodal and cathodal shock or random light paired with random cathodal shock. Neither of these controls learned the response. Thus stimulation differences can produce chemical differences.

nerve endings or synaptic vesicles, can be isolated by fractionation methods[80] but there is no way of isolating this same site in control animals in order to make a biochemical comparison. While there may be drugs that specifi- synthesis is not completely inhibited, it cannot be said that any learning or memory impairment is due solely to RNA or protein effects, since the remaining synthetic ability may suffice for learning to occur. If all protein or all RNA synthesis were turned off, it would not be surprising to see learning or memory impairment exhibited since the animal would be in a very abnormal metabolic state. Unfortunately, the metabolic inhibitors of protein and RNA do not appear to have as specific biochemical effects in higher organisms as they do in microorganisms. Puromycin and actinomycin D cause fine-structure changes in chloroplasts, nucleoli, and mitochondria in *Acetabularia*[83,84] and puromycin causes disappearance of neurotubules from axons and dendrites in the rat as well as a reduction of neurosecretory granules.[85] Puromycin also causes seizure activity in the brain.[78] Furthermore, mice that showed memory loss after injections of puromycin regained this memory when given saline injections later;[82] this result appears to indicate that puromycin blocks memory expression but not the retention of memory. The basic problem with protein inhibition is that the synthesis of all kinds of proteins is stopped and enzymes necessary in metabolic pathways will also be missing. Thus, it becomes impossible to pinpoint any particular protein as being important in learning and memory.

Experiments using drugs[78,86] that affect the nervous system may offer a potentially better approach since at least their action is limited to the nervous system. The fact that a certain drug, localized only in the nervous system, facilitates or inhibits learning and memory tells nothing, of course, about any underlying memory code. Localization of the drug with autoradiography may indicate whether neuromuscular or sensory input systems are involved. If the drug acts on these systems, it may develop the appearance of faster learning or better memory by causing the animal to move faster or see better, for example, without having any affect on the memory code. Drugs may inhibit learning by "decreasing motivation, producing ataxia, decreasing arousal and attention, impairing sensory processes, or by generally debilitating the animal,"[78, p. 158] while the drugs causing reverse effects may facilitate learning. The drugs may also affect the transmission characteristics of the nervous system, as pentylenetetrazol, picrotoxin and strychnine do,[87] or increase RNA as strychnine does.[88] Even if very specific drug binding sites involving only a few synapses are found to, say, facilitate learning, there is no guarantee the drug is bound to any chemical memory code. With radioactive drugs, the site of action, such as

Given proper controls, what can be expected from the various chemical approaches now being used? The inhibition of protein and RNA synthesis as a way of studying memory[73-82] has several drawbacks. If protein or RNA cally affect learning and memory by binding to a memory code site, we have not enough information to guide us in selecting such a drug.

It is not yet clear whether the transfer experiments where brain extracts from learned animals are injected into unlearned ones are real phenomena, since some[90-101] find the effects, and others[102-105] do not. Assuming the effects are truly chemical, and not procedural artifacts, it cannot be said that the effect directly relates to interaction with DNA, as occurs in bacterial transformation experiments, or with RNA. The fact that yeast RNA and other RNA preparations help regeneration processes[106] and may facilitate learning[107-110]* although they do not always help learning,[111] suggests that RNA from the trained animals acts like a drug that facilitates learning, or provides a metabolic pool of useful chemicals,[12] or excess RNA that can be degraded by the organisms' ribonuclease, leaving its own RNA free for other activity.[112] Intraperitoneal injections of P^{32} labelled RNA failed to reach the brain[104] indicating that any effects caused by this form of injection would not be due to RNA. It may even be contaminants, i.e. proteins, rather than the RNA in the brain injection experiments that cause the effect.[96-100] There is no denying, though, that if there is really task-specific information being transmitted, rather than a general effect, this would be most important.

The forerunners of these transfer experiments were the cannibalism experiments[113-117] where planaria that had eaten already trained ones performed better on a task than those that had eaten naive ones. One group reported that the effects could be due to procedural variables such as handling differences,[118] and another group reported negative findings.[119] Since these experiments have given way to the transfer experiments and attempts to isolate the "transfer chemical," they need not be discussed further. Related to the transfer experiments are those that show learning is retained in the regenerated animals obtained from head and tail ends of a trained planaria after it is cut in two.[120-123] The conclusion is either that the memory trace is not localized just in the brain, or a drug effect is present that facilitates learning in the severed pieces. How any of this would work is not known but it may be not unlike the way in which the transfer experiments work. The conceptual difficulty is in how the recipient animals in the transfer experiments separate the "information" to perform better on a task from all the other "information" that is presumably in the brain extract.

* Also, R. K. Siegel: Yeast RNA: effects on avoidance behavior of the neonate domestic chick. *Psychopharmacologia,* 1967, *12,* 68.

The big problem in the chemistry of learning could be that if a chemical change is responsible for memory, it may be too small to be detected by present methods. Hybridization[124] of, say, messenger RNA's from an animal that learns with those from a control suffers from the difficulty of separating any messenger RNA that may be involved in learning from the large number needed just for normal cell maintenance. Autoradiography with the electron microscope can localize radioactive molecules only to within about 500Å.[125] Many other relatively precise techniques can be listed that may be too gross for this problem, such as microelectrophoresis which deals with as little as 10^{-8} grams of protein[126] or 2.5×10^{-11} grams of RNA.[127] Clearly, such techniques cannot deal with only one protein "memory molecule" containing 500 amino acid residues, which weighs on the order of 10^{-19} grams. Even if many molecules of just one protein species were involved in memory, it would be most difficult to deal with them in the presence of thousands of other protein species. Genetics, though, does offer a way to deal with one protein species at a time and it would be theoretically possible to eliminate genetically that one species of protein, if there is one, necessary for learning. "Proof" that this protein is necessary for learning could be obtained by making antibodies to it and re-injecting it into animals to see if learning is eliminated. Immunoneurology has been considered as a relevant approach in this area[128] and as a mechanism for memory.[129] As an approach, though, the problem remains of finding the proteins that may be involved in memory. The genetic approach outlined may be impractical now, but with further research in behavioral genetics[130,131] coupled with advances in molecular genetics, it may prove to be a fruitful approach. In fact, it has been suggested that behavioral mutants with different nervous system wiring patterns can be isolated for behavioral study.[132]

CONCLUSION

We do not now know very much about the underlying basis of learning and memory. Electrical coding of memory has not been deciphered nor have structural codes been found. Protein and RNA certainly seem to be involved but they are involved in most cellular activities. Rates of synthesis of protein, ranging from 2-100 amino acids per second,[133] and messenger RNA, about one molecule per gene per second,[134] would appear too slow to account for instantaneous memory recall. Electrical events have the speed but there is no evidence as to how memory could be recalled by them. It is easy to find differences between those organisms that learn and those that do not because of slight differences in experimental procedure that occur and because of individual genetic differences that large samples will not

eliminate if proper controls are not present. The basic problem would appear to be that we do not know what characterizes a "normal" nervous system, let alone a whole organism, in terms of its ultrastructure, its electrical activity and chemical metabolism. Therefore, it is extremely difficult to separate what effect learning and memory have from what is there during an animal's normal activity and behavior.

REFERENCES

1. Applewhite, P. B. and Morowitz, H. J.: The micrometazoa as model systems for studying the physiology of memory. *Yale J. Biol. Med.*, 1966, *39*, 90-105.
2. Bruner, J. and Tauc, L.: Habituation at the synaptic level in *Aplysia*. *Nature*, 1966, *210*, 37-39.
3. Bullock, T. H. and Quarton, G. C. (Eds.): Simple systems for the study of learning mechanisms. *Neurosciences Research Program Bulletin*, 1966, *4* (No. 2).
4. Corning, W. and Ratner, R. (Eds.): *The Chemistry of Learning*. New York, Plenum Press, 1967.
5. Horridge, G. A.: Learning of leg position by the ventral nerve cord in headless insects. *Proc. roy. Soc. B*, 1962, *157*, 33-52.
6. Hoyle, G.: An isolated insect ganglion-nerve-muscle preparation. *J. exp. Biol.*, 1966, *44*, 413-427.
7. Hughes, G. M.: Neuronal pathways in the insect central nervous system. In, *The Physiology of the Insect Central Nervous System*, J. E. Treherne and J. W. L. Beament (Eds.). New York, Academic Press, 1965.
8. Kandel, E. R. and Tauc, L.: Heterosynaptic facilitation in neurons of the abdominal ganglion of *Aplysia depilans*. *J. Physiol.*, 1965, *181*, 1-27.
9. McConnell, J. V.: Learning in invertebrates. *Ann. Rev. Physiol.*, 1966, *28*, 107-136.
10. Adler, J.: Chemotaxis in bacteria. *Science*, 1966, *153*, 708-716.
11. Gaito, J. (Ed.): *Macromolecules and Behavior*. New York, Appleton-Century-Crofts, 1966.
12. ————: *Molecular Psychobiology*. Springfield, Ill., Charles Thomas, 1966.
13. John, E. R.: *Mechanisms of Memory*. New York, Academic Press, 1967.
14. Richter, D. (Ed.): *Aspects of Learning and Memory*. New York, Basic Books, 1966.
15. Schmitt, F. O. (Ed.): *Macromolecular Specificity and Biological Memory*. Cambridge, M.I.T. Press, 1962.
16. Lorente de No, R.: *A Study in Nerve Physiology*. New York, Rockefeller Institute, 1947.
17. Hebb, D. O.: *Organization of Behavior*. New York, Wiley, 1949.
18. Burns, B. D.: The production of afterbursts in isolated unanesthetized cerebral cortex. *J. Physiol.*, 1954, *125*, 427-446.
19. Verzeano, M. and Negishi, K.: Neuronal activity in cortical and thalamic networks. *J. gen. Physiol.*, 1960, *43*, Supplement, 177-195.
20. Lorente de No, R.: Circulation of impulses and memory. In, *Macromolecular Specificity and Biological Memory*, F. O. Schmitt (Ed.), Cambridge, M.I.T. Press, 1962.
21. Morrell, F.: Electrophysiological contributions to the neural basis of learning. *Physiol. Rev.*, 1961, *41*, 443-494.
22. Lewis, D. J. and Maher, B. A. Neural consolidation and electroconvulsive shock. *Psychol. Rev.*, 1965, *72*, 225-239.
23. Zinkin, S. and Miller, A. J.: Recovery of memory after amnesia induced by ECS. *Science*, 1966, *155*, 102-103.
24. Luttges, M. W. and McGaugh, J. L.: Permanence of retrograde amnesia produced by ECS. *Science*, 1967, *156*, 408-410.

25. Herz, M. J. and Peeke, H. V. S.: Permanence of retrograde amnesia produced by ECS. *Science,* 1967, *156,* 1396-1397.
26. Steele, Russell.: Animal learning and memory. In, *Aspects of Learning and Memory,* D. Richter (Ed.). New York, Basic Books, 1966.
27. Mihailovic, L., Jankovic, B., Petkovic, M., and Isakovic, K.: Effect of electroshock upon nucleic acid concentrations in various parts of the cat brain. *Experientia* (Basel), 1958, *14,* 144-145.
28. Noach, E. L., Bunk, J. J., and Wijling, A.: Influence of electroshock and phenobarbital on nucleic acid content of rat brain cortex. *Acta physiol. pharmacol. neerl.,* 1962, *11,* 54.
29. Kety, S. S., Javoy, F., Thierry, A., Julou, L., and Glowinski, J.: A sustained effect of ECS on the turnover of norepinephrine in the CNS of the rat. *Proc. nat. Acad. Sci. (Wash.),* 1967, *58,* 1249-1254.
30. Orrego, F.: Synthesis of RNA in normal and electrically stimulated brain cortex slices in vitro. *J. Neurochem.,* 1967, *14,* 851-858.
31. ———— and Lipmann, F.: Protein synthesis in brain slices. Effects of electrical stimulation and acidic amino acids. *J. biol. Chem.,* 1967, *242,* 665.
32. Gerard, R. W.: Biological roots of psychiatry. *Science,* 1955, *122,* 225-230.
33. Pearlman, C. A., Sharpless, S. K., and Jarvik, M. E.: Retrograde amnesia produced by anesthetic and convulsant agents. *J. comp. physiol. Psychol.,* 1961, *54,* 109.
34. Andjus, R. K., Knopfelmacher, F., Russell, R. W., and Smith, A. U.: Some effects of severe hypothermia on learning and retention. *Quart. J. exp. Psychol.,* 1956, *8,* 15-23.
35. Mrosovsky, N.: Retention and reversal of conditioned avoidance following severe hypothermia. *J. comp. physiol. Psychol.,* 1963, *56,* 811-813.
36. Ransmeier, R. E. and Gerard, R. W.: Effects of temperature, convulsion and metabolic factors on rodent memory and EEG. *Amer. J. Physiol.,* 1954, *179,* 663-664.
37. Meldrum, B. S.: Electrical signals in the brain and the cellular mechanism of learning. In, *Aspects of Learning and Memory,* D. Richter (Ed.). New York, Basic Books, 1966.
38. Dern, H. and Walsh, J. B.: Analysis of complex waveforms. In, *Physical Techniques in Biological Research,* W. L. Nastuk (Ed.). New York, Academic Press, 1963, Volume 6, Part B.
39. Milsum, J. H.: *Biological Control System Analysis.* New York, McGraw-Hill, 1966, p. 354.
40. Stevens, C. F.: *Neurophysiology: A Primer.* New York, Wiley, 1966, p. 18.
41. Gerstein, G. and Kiang, N. Y. S.: An approach to the quantitative analysis of electrophysiological data from single neurons. *Biophys. J.,* 1960, *1,* 15-28.
42. Moore, G. P., Perkel, D. H., and Segundo, J. P.: Statistical analysis and functional interpretation of neuronal spike data. *Ann. Rev. Physiol.,* 1966, *28,* 493-522.
43. Rodieck, R. W., Kiang, N. Y. S., and Gerstein, G. L.: Some quantitative methods for the study of spontaneous activity of single neurons. *Biophys. J.,* 1962, *2,* 351-368.
44. Pumphrey, R. J. and Rawdon-Smith, A. F.: Synaptic transmission of nervous impulses through the last abdominal ganglion of the cockroach. *Proc. roy. Soc. B, Proceedings,* 1937, *122,* 106-118.
45. Willows, A. O. D.: Behavioral acts elicited by stimulation of single, identifiable brain cells. *Science,* 1967, *157,* 570-574.
46. Katz, B.: *Nerve, Muscle and Synapse.* New York, McGraw-Hill, 1966, p. 169.
47. Eccles, J. C.: *The Physiology of Synapses.* Berlin, Springer-Verlag, 1964.
48. Aidley, D. J.: A suggestion regarding the cellular basis of learning. *J. theor. Biol.,* 1966, *11,* 343-345.
49. Dolley, D. H.: The morphology of functional depression in nerve cells and its significance for the normal and abnormal physiology of the cell. *J. med. Res.,* 1913, *29,* 65-129.
50. Kocher, R. A.: The effect of activity on the histological structure of nerve cells. *J. comp. Neurol.,* 1916, *26,* 341-357.

51. Edstrom, J. E.: Effects of increased motor activity on the dimensions and the staining properties of the neuron soma. *J. comp. Neurol.*, 1957, *107*, 295-304.
52. DeRobertis, E. and Franchi, C. M.: Electron microscopic observations on synaptic vesicles in synapses of the retinal rods and cones. *J. biophys. biochem. Cytol.*, 1956, *2*, 307-318.
53. Mountford, S.: Effects of light and dark adaptation in the vesicle populations of receptor-bipolar synapses. *Exp. Cell Res.*, 1963, *9*, 403-418.
54. Appel, S. H., Davis, W., and Scott, S.: Brain polysomes: response to environmental stimulation. *Science*, 1967, *157*, 836-838.
55. DeRobertis, E. and Ferreira, A.: Submicroscopic changes of the nerve endings in the adrenal medulla after stimulation of the splanchnic nerve. *J. biophys. biochem. Cytol.*, 1957, *3*, 611-614.
56. Elul, R.: Dependence of synaptic transmission on protein metabolism of nerve cells: a possible electrokinetic mechanism of learning? *Nature*, 1966, *210*, 1127-1131.
57. Larramendi, L. M. H., Fickenscher, L., and Lemkey-Johnston, N.: Synaptic vesicles of inhibitory and excitatory terminals in the cerebellum. *Science*, 1967, *156*, 967-969.
58. Altman, J.: Autoradiographic and histological studies of postnatal neurogenesis. *J. comp. Neurol.*, 1966, *128*, 431-473.
59. Bennett, E. L., Diamond, M. C., Krech, D., and Rosenzweig, M. R.: Chemical and anatomical plasticity of brain. *Science*, 1964, *146*, 610-619.
60. Diamond, M. C., Law, F., Rhodes, H., Lindner, B., Rosenzweig, M. R., Krech, D., and Bennett, E. L.: Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J. comp. Neurol.*, 1966, *128*, 117-125.
61. Altman, J. and Das, G. D.: Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. *Nature*, 1964, *204*, 1161-1163.
62. Jahn, T. L. and Bovee, E. C.: Motile behavior of protozoa. In, *Research in Protozoology*, Vol. 1. T. Chen (Ed.). New York, Pergamon Press, 1967.
63. Pevzner, L. Z.: Nucleic acid changes during behavioral events. In, *Macromolecules and Behavior*, J. Gaito (Ed.). New York, Appleton-Century-Crofts, 1966.
64. Zemp, J. W., Wilson, J. E., Schlesinger, K., Boggan, W. O., and Glassman, E.: Brain function and macromolecules, I. Incorporation of uridine into RNA of mouse brain during short-term training experience. *Proc. nat. Acad. Sci. (Wash.)*. 1966, *55*, 1423-1431.
65. Mason, J. W., Brady, J. V., Polish, E., Bauer, J. A., Robinson, J. A., Rose, R. M., and Taylor, E. D.: Patterns of corticosteroid and pepsinogen change related to emotional stress in the monkey. *Science*, 1961, *133*, 1596-1598.
66. Plotnikoff, N.: Magnesium pemoline: enhancement of learning and memory of a conditioned avoidance response. *Science*, 1966, *151*, 703-704.
67. Beach, G. and Kimble, D. P.: Activity and responsitivity in rats after magnesium pemoline injections. *Science*, 1967, *155*, 698-701.
68. Hyden, H. and Eghyhazi, E.: Nuclear RNA changes of nerve cells during a learning experiment in rats. *Proc. nat. Acad. Sci. (Wash.)*, 1962, *48*, 1366-1373.
69. ————.: Changes in RNA content and base composition in cortical neurons of rats in a learning experiment involving transfer of handedness. *Proc. nat. Acad. Sci. (Wash.)*, 1964, *52*, 1030-1035.
70. ————.: Glial RNA changes during a learning experiment with rats. *Proc. nat. Acad. Sci. (Wash.)*, 1963, *49*, 618-624.
71. Hyden, H. and Lange, P. W.: A differentiation in RNA response in neurons early and late during learning. *Proc. nat. Acad. Sci. (Wash.)*, 1965, *53*, 946-952.
72. Kuffler, S. W. and Nicholls, J. G.: The physiology of neuroglial cells. *Ergebn. Physiol.*, 1966, *57*, 1-90.
73. McGaugh, J. L. and Petrinovich, L. F.: Effects of drugs on learning and memory. *Int. Rev. Neurobiol.*, 1965, *8*, 139-196.

74. Agranoff, B. W., Davis, R. E., and Brink, J. J.: Memory fixation in the goldfish. *Proc. nat. Acad. Sci. (Wash.)*, 1965, *54*, 788-793.
75. Brink, J. J., Davis, R. E., and Agranoff, B. W.: Effects of puromycin, acetoxycycloheximide and actinomycin D on protein synthesis in goldfish brain. *J. Neurochem.*, 1966, *13*, 889-896.
76. Barondes, S. H. and Cohen, H. D.: Puromycin effect on successive phases of memory storage. *Science*, 1966, *151*, 594-595.
77. Cohen, H. D. and Barondes, S. H.: Further studies of learning and memory after intracerebral actinomycin D. *J. Neurochem.*, 1966, *13*, 207-211.
78. ————.: Puromycin effect on memory may be due to occult seizures. *Science*, 1967, *157*, 333-334.
79. Barondes, S. H. and Cohen, H. D.: Delayed and sustained effective of acetoxycycloheximide on memory in mice. *Proc. nat. Acad. Sci. (Wash.)*, 1967, *58*, 157-164.
80. Flexner, L. B., Flexner, J. B., and Roberts, R. B.: Stages of memory in mice treated with acetoxycycloheximide before or immediately after learning. *Proc. nat. Acad. Sci. (Wash.)*, 1966, *56*, 730-735.
81. ————: Memory in mice analyzed with antibiotics. *Science*, 1967, *155*, 1377-1383.
82. Flexner, J. B. and Flexner, L. B.: Restoration of expression of memory lost after treatment with puromycin. *Proc. nat. Acad. Sci. (Wash.)*, 1967, *57*, 1651-1654.
83. Boloukhere-Presburg, M.: Effet de l' Actinomycine D sur l' ultrastructure des chloroplastes et du noyau d' *Acetabularia mediterranea*. *J. Microscopie*, 1965, *4*, 363-372.
84. ————: Effets de la puromycine sur l' ultrastructure d' *Acetabularia mediterranea*. *J. Microscopie*, 1966, *5*, 619.
85. Zambrano, D. and de Robertis, E.: Ultrastructural aspects of the inhibition of neurosecretion by puromycin. *Z. Zellforsch.*, 1967, *76*, 458-470.
86. Gollub, L. R. and Brady, J. V.: Behavioral pharmacology. *Ann. Rev. Pharmacol.*, 1965, *5*, 235-262.
87. Irwin, S. and Benuazizi, A.: [illegible]ntylenetetrazol enhances memory function. *Science*, 1966, *152*, 100-102.
88. Carlini, G. R. S. and Carlini, . .: Effects of strychnine and cannabis satira (marijuana) on the nucleic acid content in the brain of the rat. *Medicina et Pharmacologia Experimentalis*, 1965, *12*, 21-26.
89. Whittaker, V. P.: The application of subcellular fractionation techniques to the study of brain function. *Prog. Biophys. molec. Biol.*, 1965, *15*, 39-96.
90. Zelman, A., Kabat, L., Jacobson, A., and McConnell, J. V.: Transfer of training through injection of "conditioned" RNA into untrained planarians. *Worm Runner's Digest*, 1963, *5*(1), 14.
91. Fjerdingstad, E. J., Nissen, T., and Roigaard-Petersen, H. H.: Effect of RNA extracted from the brain of trained animals on learning in rats. *Scand. J. Psychol.*, 1965, *6*, 1-6.
92. Ungar, G. and Oceguera-Navarro, C.: Transfer of habituation by material extracted from brain. *Nature*, 1965, *207*, 301-302.
93. ———— and Irwin, L. N.: Transfer of acquired information by brain extracts. *Nature*, 1967, *214*, 453.
94. Babich, F. R., Jacobson, A., Bubash, S., and Jacobson, A.: Transfer of a response to naive rats by injection of RNA extracted from trained rats. *Science*, 1965, *149*, 656-657.
95. Jacobson, A.: Cross species transfer of training: effect of RNA from hamsters on rat behavior. *Proc. nat. Acad. Sci. (Wash.)*, 1965, *54*, 1299-1302.
96. ————, Babich, F. R., Bubash, S., and Jacobson, A.: Differential-approach tendencies produced by injection of RNA from trained rats. *Science*, 1965, *150*, 636-637.
97. ————, Fried, C., and Horowitz, S. D.: Planarians and memory. *Nature*, 1966, *209*, 599-601.

98. Rosenblatt, F., Farrow, J. T., and Rhine, S.: The transfer of learned behavior from trained to untrained rats by means of brain extracts, I, II. *Proc. nat. Acad. Sci. (Wash.)*, 1966, *55*, 548-555, 787-792.
99. Rosenblatt, F., Farrow, J. T., and Herblin, W. F.: Transfer of conditioned responses from trained rats to untrained rats by means of a brain extract. *Nature*, 1966, *209*, 46-48.
100. ———— and Miller, R. G.: Behavioral assay procedures for transfer of learned behavior by brain extracts, I, II. *Proc. nat. Acad. Sci. (Wash.)*, 1966, *56*, 1423-1430, 1683-1688.
101. Dyal, J. A., Golub, A. M., and Marrone, R. L.: Transfer effects of intraperitoneal injection of brain homogenates. *Nature*, 1967, *214*, 720-721.
102. Gross, C. G. and Carey, F. M.: Transfer of learned response by RNA injection: failure of attempts to replicate. *Science*, 1965, *150*, 1749.
103. Gordon, M. W., Deanin, G. G., Leonhardt, H. L. and Gwinn, R. H.: RNA and memory: a negative experiment. *Amer. J. Psychiat.*, 1966, *122*, 1174.
104. Luttges, M., Johnson, T., Buck, C., Holland, J., and McGaugh, J.: An examination of "transfer of learning" by nucleic acid. *Science*, 1966, *151*, 834-837.
105. Byrne, W. L., *et al.*: Memory transfer. *Science*, 1966, *153*, 658-659.
106. Batkin, S.: The effect of RNA on the recovery of spinal-sectioned carp. *Proc. nat. Acad. Sci. (Wash.)*, 1966, *56*, 1689-1691.
107. Cameron, D. E., Sved, S., Solyom, L., Wainrib, B., and Barik, H.: Effects of RNA on memory deficit in the aged. *Amer. J. Psychiat.*, 1963, *120*, 320-325.
108. Cook, L., Davidson, A., Davis, D., Green, H. and Fellows, E. J.: RNA: effect on conditioned behavior in rats. *Science*, 1963, *141*, 268-269.
109. Goren, C.: RNA: influence on the maze-learning ability of rats. *Worm Runner's Digest*, 1965, *7* (2), 28-31.
110. Brown, H.: Effect of RNA on reversal of a probability matching problem in pigeons. *Psychol. Record*, 1966, *16*, 441.
111. Cohen, H. D. and Barondes, S. H.: Lack of effect of a highly purified yeast RNA preparation on maze learning. *Psychopharmacologia (Berl.)*, 1966, *8*, 375.
112. Kral, V. A. and Sved, S.: Paper presented at symposium on Nucleic Acids and Behavior, Chicago, 1963.
113. McConnell, J. V., Jacobson, R., and Humphries, B. M.: The effects of ingestion of conditioned planaria on the response level of naive planaria: a pilot study. *Worm Runner's Digest*, 1961, *3*, 41-47.
114. McConnell, J. V.: Memory transfer through cannibalism in planaria. *J. Neuropsychiat.*, 1962, *3*, Supplement 1, 542-548.
115. Kabat, L.: Transfer of training through ingestion of conditioned planarians by unconditioned planarians. *Worm Runner's Digest*, 1964, *6* (1), 23-27.
116. Fried, C. ad Horowitz, S.: Contraction: a learnable response. *Worm Runner's Digest*, 1964, *6* (2), 3-6.
117. Feldman, E. J. and Capretta, P. J.: Post conditioning delay and memory transfer through cannibalism. *Worm Runner's Digest*, 1965, *7* (2), 35-41.
118. Hartry, A., Keith-Lee, P., and Morton, W. D.: Planaria: memory transfer through cannibalism re-examined. *Science*, 1964, *146*, 274-275.
119. Picket, J. B. E., Jennings, L. B., and Wells, P. H.: Influence of RNA and victim training on maze learning by cannibal planarians. *Amer. Zoologist*, 1964, *4*, 158.
120. McConnell, J. V., Jacobson, A. L., and Kimble, D. P.: The effects of regeneration upon retention of a conditioned response in the planarian. *J. comp. physiol. Psychol.*, 1959, *52*, 1-5.
121. Westerman, R. A.: Somatic inheritance of habituation of responses to light in planarians. *Science*, 1963, *140*, 676-677.
122. Corning, W. C. and John, E. R.: Effect of ribonuclease on retention of conditioned response in regenerated planarians. *Science*, 1961, *134*, 1363-1365.
123. Corning, W. C.: Retention of a position discrimination after regeneration in planarians. *Psychonomic Sci.*, 1966, *5*, 17-18.
124. Gillespie, D. and Spiegelman, S.: A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. molec. Biol.*, 1965, *12*, 829-842.

125. Caro, L.: Progress in high-resolution autoradiography. *Progr. Biophys. molec. Biol.*, 1966, *16*, 171-190.
126. Hyden, H., Bjurstam, K. and McEwen, B.: Protein separation on the cellular level by micro-disc electrophoresis. *Analyt. Biochem.*, 1966, *17*, 1-15.
127. Edstrom, J. E.: Microextraction and microelectrophoresis for determination and analysis of nucleic acids in isolated cellular units. In, *Methods in Cell Physiology*, Vol. 1. D. M. Prescott (Ed.). New York, Academic Press, 1964.
128. Immuno-Neurology. *Neurosciences Research Program Bulletin*, 1965, *3*, No. 1, 1-26.
129. Mekler, L. B.: Mechanism of biological memory. *Nature*, 1967, *215*, 481.
130. McClearn, G. E. and Meredith, W.: Behavioral genetics. *Ann. Rev. Psychol.*, 1966, *17*, 515-550.
131. Ewing, A. W. and Manning, A.: Evolution and genetics of insect behavior. *Ann. Rev. Entomol.*, 1967, *12*, 471-494.
132. Benzer, S.: Behavioral mutants of Drosophila isolated by countercurrent distribution. *Proc. nat. Acad. Sci. (Wash.)*, 1967, *58*, 1112-1119.
133. Mahler, H. R. and Cordes, E. H.: *Biological Chemistry*. New York, Harper and Row, 1966.
134. Hartman, P. E. and Suskind, S. R.: *Gene Action*. Englewood Cliffs, N. J., Prentice-Hall, 1965.